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Myrtus communis in Europe threatened by the pandemic and South African strains of the myrtle rust pathogen *Austropuccinia psidii* (Sphaerophragmiaceae, Pucciniales)

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Abstract

Austropuccinia psidii, the causal agent of myrtle rust, has emerged as a significant threat to Myrtaceae in planted and natural woody ecosystems. The first detection of *A. puccinia* in South Africa was from severely infected ornamental *Myrtus communis*. This raised concern that *M. communis*, the sole Myrtaceae species native to Europe and an important component of vegetation in Mediterranean regions, could be threatened by the rust. In light of the potential threat to this unique species, seed was collected from 12 Italian provenances of *M. communis*, including mainland and island (Sardinia and Sicily) populations. We assessed the susceptibility of these provenances to both the pandemic and South African strains of *A. psidii*. In Colombia, where the pandemic strain of *A. psidii* is native, seedlings rapidly became infected by natural inoculum. In South Africa, a preliminary screening of seedlings by artificial inoculation with a single-uredinium isolate produced high levels of disease. Finally, plants of each of the 12 provenances were planted and monitored in Florence, Italy. To date, these showed no signs of disease, but will continue to be monitored. This study highlights the significant threat that both the pandemic and South African strains of *A. puccinia* pose to *M. communis* in Europe.

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Keywords
biosecurity, emerging diseases, invasive forest pathogens, microbial invasions, sentinel plants

Austropuccinia psidii (G. Winter) Beenken (basionym *Puccinia psidii*, Sphaerophragmiaceae, Pucciniales) has emerged as an important invasive plant pathogen, threatening Myrtaceae in planted and natural woody ecosystems globally (Coutinho et al. 1998; Carnegie et al. 2016; Roux et al. 2016; Beenken 2017). Originating in South America (Coutinho et al. 1998; Glen et al. 2007), the pathogen is the causal agent of a rust disease with common names including guava rust, eucalyptus rust, ʻōhiʻa rust and myrtle rust. *Austropuccinia psidii* has a wide host range of over 480 species of Myrtaceae (Soewarto et al. 2019) and currently represents the major biosecurity threat for the Myrtaceae family.

In 1998, *A. psidii* was added to the EPPO Alert List, based on the threat the pathogen posed to *Eucalyptus*, which are considered important trees in Mediterranean countries. In 2003, however, the listing was deleted, following the conclusion from PRA (02-9886 & 9891) that the risk was low due to the climatic requirements of the rust (EPPO 2003). In the two decades that have passed since this decision, *A. psidii* has invaded diverse geographic regions including Africa, Asia-Pacific and Oceania (Carnegie et al. 2010; Giblin 2013; Roux et al. 2013, McTaggart et al. 2016; du Plessis et al. 2019), with evidence that the pathogen is able to spread rapidly and results in devastating impacts (Carnegie and Pegg 2018).

A globally-important new record of *A. puccinia* was when it was first detected in South Africa on ornamental *Myrtus communis* Linn. (Roux et al. 2013). *Myrtus communis* is native to southern Europe, North Africa and West Asia. It is the sole Myrtaceae species native to Europe and an important component of vegetation in Mediterranean regions. In addition to its occurrence in natural ecosystems, it is also widely cultivated for its ornamental value and aromatic properties. In particular, its berries, leaves, seeds and essential oils are natural sources of several nutrients and bioactive compounds and, in the Italian region of Sardinia, berries are used in the production of an important local liqueur, '*Mirto di Sardegna*' (Sumbul et al. 2011; Giampieri et al. 2020). Observations from South Africa (Roux et al. 2013, 2016) indicated that *A. psidii* infection resulted in severe disease development, highlighting the threat posed to this unique plant. This prompted the question as to the relative threat of *A. psidii* to *M. communis* in Europe.

Seeds were collected from 12 Italian provenances of *M. communis*, including mainland and island (Sardinia and Sicily) populations (Table 1). Seeds were cleaned and prepared at the Institute for Sustainable Plant Protection - National Research Council (IPSP-CNR) facilities in Florence, Italy, before being sent to Colombia and South Africa, to be screened for susceptibility to the pandemic and South African strains of *A. psidii*, respectively. In Colombia, seeds were sown in June 2019 at the phytopathology laboratory of Smurfit Kappa (Restrepo, Valle del Cauca: 3.855278, -76.5075). Germination rates varied from 23–100%, with the number of seedlings per provenance varying from 170–920. Plants were maintained outside; however, these rapidly became infected by natural inoculum present on planted *Syzygium jambos* (L.)

Alston. A known susceptible host of *A. psidii*, the identification of the pandemic strain of *A. psidii* was previously confirmed on this stand of *S. jambos* by Granados et al. (2017). Yellow masses of urediniospores were frequently observed on shoots and young leaves and stems of the *M. communis* plants, with telia also occasionally developing. By March 2022 (33 months after germination), extensive mortality had occurred, due to repeated natural infection (Fig. 1). Mortality varied by provenance, ranging from 85.3–100% mortality (mean = 95.4%).

In South Africa, seeds were sown in February 2021 in a phytotron maintained under controlled conditions (ca. 25 °C, 75%–85% relative humidity, 12 hr natural daylight/12 hr night) at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (Pretoria, Gauteng: -25.755537, 28.235440). Artificial inoculation of a selection of seedlings (seven replicate plants from nine provenances) was conducted in September 2021 (7 months after germination). The inoculation was carried out using a single pustule isolate of the South African strain, originally collected from *Eugenia natalitia* Sond. (syn. *Eugenia capensis* subsp. *natalitia*) and mass produced on seedlings of *S. jambos*, as described by Roux et al. (2016). The plants were assessed for disease symptoms after 14 days. Symptoms started to develop within a few days following inoculation. After 14 days, all individuals (n = 63) showed typical symptoms of infection by *A. psidii* (Fig. 1). Pustules with uredinia developed on young leaves and stems of nearly all plants, with telia also developing on many individuals.

Plants of each of the 12 provenances were planted at the experimental facility of the IPSP-CNR in Florence, Italy (43.771944, 11.177500). The planting was established in line with the "Sentinel Planting" approach, to optimise early detection of alien pests and pathogens (for detail, see Migliorini et al. 2022). Monitoring of these plants is ongoing; to date they remain disease-free.

The results arising from the natural infection in Colombia and a preliminary screening using artificial inoculation in South Africa, indicate that a wide range of provenances of *M. communis* are highly susceptible to both the pandemic and South African strains of *A. psidii*. The 2003 decision by EPPO to delist this pathogen was based on the finding that the risk of pathogen establishment in Europe was low due to

Table 1. Italian provenances of *Myrtus communis* screened for susceptibility to *Austropuccinia psidii*.

Region	Municipality and Province	Location
Sardinia	Sinnai (Cagliari)	Pineta di Sinnai
Sardinia	Villa Cidro (Mediocampidano)	Cannamenda
Sicily	Buccheri (Siracusa)	CDA: Santa Maria
Sicily	Militello Rosmarino (Messina)	Luco
Sicily	Eraclea Minoa (Agrigento)	CDA: Borgo Bonsignore
Sicily	Caronia (Messina)	CDA: Pagliarotta
Sicily	Noto (Siracusa)	RNO: Vendicari
Sicily	Ragusa	CDA: Cava Dei Modicani
Sicily	Randazzo (Catania)	CDA: La Guardia
Sicily	Buseto Palizzolo (Trapani)	CDA: Scorace
Tuscany	Pisa	San Rossore
Tuscany	Cecina (Livorno)	Cecina

climatic requirements. However, in the two decades that have followed, *A. psidii* has invaded many new regions. The spread of the pathogen into warm to cool temperate climates in Australia and New Zealand has provided opportunities to study the biology and epidemiology of *A. psidii* under conditions different to those found in the tropical and subtropical areas from which the pathogen originates. For example, Beresford et al. (2020) demonstrated that the uredinial stage of *A. psidii* is capable of overwintering (in a latent phase) in cool temperate climates. A CLIMEX model developed by Kriticos et al. (2013) showed *A. psidii's* preference for moist climates with moderate temperatures throughout the wet tropics and sub-tropics; however, the model also identified some cool regions with a mild Mediterranean climate as climatically suitable areas. It is likely that revising the model, including distribution data from more recently invaded areas, will demonstrate an increase in the range of areas suitable for the pathogen to establish.

The original EPPO listing was based on the threat *A. psidii* posed to *Eucalyptus* in Mediterranean areas of Europe. While the pathogen continues to threaten this economically important species (Hakamada et al. 2022), there are growing concerns regarding the threat that *A. psidii* poses to native ecosystems, particularly in regions with high levels of Myrtaceae diversity and endemism. A number of Australian species are now threatened with biological extinction, as a direct result of continued infection

Figure 1. top – natural infection of ornamental *Myrtus communis* in Cape Town (Western Cape) (*Austropuccinia psidii* South African strain) (left); natural infection of Italian provenances of *M. communis* in Colombia (*A. psidii* pandemic strain) (centre and right); bottom – disease development and prolific production of urediniospores14 days after artificial inoculation (*A. psidii* South African strain).

(Pegg et al. 2017; Makinson et al. 2020). We believe the findings communicated here should prompt a re-evaluation of the risk *A. psidii* presents to Europe, taking into consideration the demonstrated threat that both the pandemic and South African strains of *A. puccinia* pose to *M. communis*.

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